33. The method of claim 31 wherein the transducer is applied to the tissue or cells during surgery.

Remarks

Claims 1-31 were pending and have been amended. Claims 32 and 33 have been added. Support for the new claims is found at page 11, lines 14-27.

Objection to the Specification

The specification was objected to as lacking antecedent basis for the ranges in claims 14 and 20. Since these claims appeared in the application as originally filed and therefore form a part of the specification, the specification has been amended to reference the claimed ranges, defined by claims 14, 19 and 20 (claim 20 actually being a subset of the language of claim 19), at pages 6 and 8.

The abstract has been amended to delete the term "means" and to insert --devices-- in place thereof.

Rejection Under 35 U.S.C. § 112, second paragraph

Claims 9, 17, 19-22, and 26 were rejected under 35 U.S.C. § 112, second paragraph, as being indefinite. Applicants respectfully traverse this rejection to the extent that it is applied to the claims as amended.

Claims 9, 17, 19, 20, 21, 22 and 22 have been amended to correct antecedent basis and to clarify the claimed method steps, in response to the examiner's concerns. Claim 26 has been amended to recite the structural elements required for the method of claim 1, which is no longer referenced in claim 26.

Rejection Under 35 U.S.C. § 102

Claims 1, 3-5, 8-12, 14, 15, 17, 18, 23, 25, and 26 were rejected under 35 U.S.C. § 102(e) as being anticipated by U.S. Patent No. 5,656,016 to Ogden ("Ogden"). Claims 1-3, 5, 7, 14, 15, 17, 18, 23, 25, and 26 were rejected under 35 U.S.C. § 102(b) as being anticipated by Tachibana et al., Enhancement of cell killing of HL-60 cells by ultrasound in the presence of the photosensitizing drug Protofrin II", *Cancer Lett.* 72(3):195-199 (1993) ("Tachibana"). Claims 27-28, 30, and 31, and 26 were rejected under 35 U.S.C. § 102(b) as being anticipated by U.S. Patent No. 5,445,611 to Eppstein et al. ("Eppstein"). Applicants respectfully traverse this rejection to the extent that it is applied to the claims as amended.

Ogden

Ogden discloses a sonophoretic drug delivery system. The device may include a feedback phase-tracking loop, which ensures that the frequency of the transducer matches the frequency of the amplified input signal. Ogden teaches that this feedback loop is necessary to ensure the maximum utilization of the amplified signal and its conversion to mechanical energy. (see col. 4, lines 17-26) Ogden discloses that the device may include a biological feedback system *to sense the amount of agen*t received by the body *or body temperature* and control the delivery of additional drug. (see col. 6, lines 36-45). Nowhere does Ogden teach or suggest that *a property of the acoustic energy can be measured* and *then used to modify further application of acoustic energy*. Thus, Ogden does not teach every element of the claimed methods, and claims 1, 3-5, 8-12, 14, 15, 17, 18, 23, 25, and 26, as amended, are novel in view of Ogden.

Tachibana, et al.

Tachibana, et al., enhances killing of tumor cells by a drug, photofrin II, a porphyrin, by increaing uptake of the drug using ultrasound. The ultrasound was applied at one of three different intensities. Cell viability was then measured. This is not the claimed method. There is no "real time" feedback contemplated nor demonstrated, allowing adjustment of the ultrasound properties at the time of application. Moreover, there is no measurement of any acoustical property, as clearly required by the amended claims. Therefore Tachibana, et al., fails to disclose the elements of claims 1-3, 5, 7, 14, 15, 18, 23, 25, or 26.

Eppstein

Eppstein teaches using ultrasound to alter drug delivery or transport of analyte from cells in the skin, when the ultrasound is applied transdermally. Eppstein does not teach altering the permeability or viability of cells at a blood vessel, tissue or organ, either by direct application of the ultrasound to the membrane, vessel, tissue or organ, or by indirect application via the skin or a mucosal membrane. Therefore Eppstein does not anticipate the method of claims 27, 28, 30 or 31.

Rejection Under 35 U.S.C. § 103

Claims 1-6, 8-18, and 23-26 were rejected under 35 U.S.C. § 103(a) as obvious over U.S. Patent No. 5,636,632 to Bommannan, et al. ("Bommannan"). Claims 1-5, 8-18, 23-26, and 29 were rejected under35 U.S.C. § 103(a) as obvious over Eppstein. Claims 2, 13, 16, and 24 were rejected under 35 U.S.C. § 103(a) as obvious over Ogden. Applicants respectfully traverse this rejection to the extent that it is applied to the claims as amended.

Bommannan

Bommannan describes application of ultrasound to enhance drug delivery. Bommannan fails to disclose measurement of an acoustic parameter in order to modify the ultrasound application. Even as to the properties that are measured, these are static measurements; they are not used to modify the applied ultrasound.

Ogden and Eppstein are discussed above. Accordingly, alone or in combination with any of Ogden or Eppstein, there is no teaching that one should measure an acoustic parameter in order to modify the ultrasound being applied. It is well established that for a rejection under section 103, the prior art must not only disclose the claimed elements, but teach one skilled in the art to combine and modify the claimed elements as applicants have done. The prior art simply fails to do so. There is no recognition in any of the art cited by the examiner that acoustic properties can be monitored and then used to provide feedback to control the properties of the applied ultrasound to thereby modify cell permeability or viability. Therefore the methods of claims 1-25, the device of claim 26, and the method of claim 29 are not obvious.

Marked Up Version of Amended Claims
Pursuant to 37 C.F.R. § 1.121(c)(1)(ii)

1. (Amended) A method for altering permeability, cell viability or structural integrity of biological materials comprising

(a) administering acoustic energy to the biological materials at one or more frequencies;

(b) measuring [the effect of the acoustic energy or] a property of the acoustic energy at the time of or subsequent to the initial application of the acoustic energy; and

(c) using the measurement obtained in step (b) to modify continued or subsequent application of acoustic energy to the biological materials.

2. The method of claim 1 wherein the property of the acoustic energy being measured in step b is one or more properties selected from the group consisting of pressure at one or more frequencies, energy input at one or more frequencies, and length of time the acoustic energy is administered.

3. The method of claim 1 wherein the acoustic energy is effective to alter permeability of the biological materials to a chemical or biological agent selected from the group consisting of peptides, proteins, sugars, polysaccharides, nucleotides, polynucleotide molecules, synthetic organic compounds, synthetic inorganic compounds, and combinations and aggregates thereof.

4. The method of claim 3 wherein the agent is in a form selected from the group consisting of cells or virus particles, nano or microparticles, liposomes or other lipid vesicles or emulsions.

5. The method of claim 3 wherein the chemical or biological agent is delivered to cells or tissue.

6. The method of claim 3 wherein the chemical or biological agent is detected or quantitated, further comprising

removing biological fluid or molecules simultaneously, previously, or subsequently to the application of acoustic energy, and

assaying the biological fluid or molecules to detect or quantitate the chemical or biological agents.

7. The method of claim 1 wherein the acoustic energy is administered to kill cells.

8. The method of claim 1 wherein the biological materials are made more permeable by the exposure to acoustic energy.

9. (amended) The method of claim 8 wherein the<u>biological materials are made</u> [increased permeability is] partially or completely [reversible] <u>reversibly permeable</u>.

10. The method of claim 1 wherein the biological materials are biological membranes.

11. The method of claim 1 wherein the biological material is skin.

12. The method of claim 1 wherein the acoustic energy is applied to biological materials in an amount effective to disaggregate or dissociate the materials.

13. The method of claim 1 wherein the biological materials are blood vessels.

14. The method of claim 1 wherein the acoustic energy is applied at a frequency between 1 kHz and 10 MHz.

15. The method of claim 1 wherein the acoustic energy is ultrasound.

16. The method of claim 1 wherein the acoustic energy is applied at a peak positive pressure of up to 100 atmospheres.

17. (amended) The method of claim 1 wherein the [application of] acoustic energy [causes] is applied under conditions to effect cavitation within or on the surface of the biological materials.

18. The method of claim 1 further comprising administering an agent to enhance transport within or permeability of the biological materials.

19. (Amended) The method of claim 1 wherein the property of the acoustic energy that is measured [acoustic energy or pressure] is measured at one or more frequencies other than the frequency or frequencies at which the acoustic energy is applied.

20. (Amended) The method of claim 1 wherein the property of the acoustic energy that is measured [acoustic energy or pressure] is measured at a frequency or frequencies corresponding to integer multiples of one-half or one-fourth of the frequency applied.

21. (Amended) The method of claim 1 wherein the acoustic energy is measured at one or more frequencies in the acoustic spectrum which do not correspond to peaks in the acoustic spectrum and are taken from the broadband signal of the acoustic spectrum.

22. (Amended) The method of claim 19 wherein the acoustic energy measurement is analyzed using a mathematical algorithm, [such as] selected from the group consisting of Fourier Transform [or the] and Fast Fourier Transform.

23. The method of claim 1 wherein the application of the acoustic energy is modified by changing an acoustic parameter selected from the group consisting of pressure, energy, frequency, pulse length, total exposure time, duty cycle, and combinations thereof.

24. The method of claim 1 wherein the application of the acoustic energy is modified by changing a non-acoustic parameter selected from the group consisting of temperature, fluid gas content, administration rate of molecules to be transported, sample collection rate, device position, and combinations thereof.

25. The method of claim 1 wherein the application of the acoustic energy input is modified by interrupting the application.

26. (Amended) A device [for use in the method of any of claims 1-25] comprising

(a) means for administering acoustic energy to biological materials at one or more frequencies;

(b) means for measuring a property of the acoustic energy at the time of or subsequent to the initial application of the acoustic energy; and

(c) means for using the measurement of the property of the acoustic energy to modify continued or subsequent application of acoustic energy to the biological materials.

27. (Amended) A method for altering cell viability or transport of chemical or biological agents into or through [biological materials or cell viability] an internal organ, internal tissue or vessels in a human or other animal using acoustic energy[, wherein the biological materials or cells are at a site distant from the site of application of the acoustic energy], comprising:

administering acoustic energy at one or more frequencies by applying a transducer to a [first] site on the human or other animal;

wherein the acoustic energy alters transport or cell viability [at a second site in the human or other animal distant from the first site] at an internal organ, tissue or vessel.

28. (Amended) The method of claim 27 wherein the [first site is] acoustic energy is applied to the skin or a mucosal membrane and [the second site is] alters transport or cell viability at an internal organ, tissue or vessel.

29. (Amended) The method of claim 27 wherein the [cells are] acoustic energy alters transport or cell viability of tumor cells.

30. (Amended) The method of claim 27 wherein the acoustic energy alters transport into or out of the cells of molecules selected from the group consisting of therapeutic, prophylactic and diagnostic agents.

31. (Amended) The method of claim 27 wherein the transducer is directly applied to a tissue [other than the biological materials where transport or cell viability is to be altered] using invasive or minimally invasive means.

Please add the following new claims 32 and 33.

32. The method of claim 31 wherein the transducer is applied to a blood vessel using a catheter.

33. The method of claim 31 wherein the transducer is applied to the tissue or cells during surgery.

Clean Version of Amended Specification Paragraphs
Pursuant to 37 C.F.R. § 1.121(b)(1)(iii)

ATL1 #474664 v1

Detailed Description Of The Disclosure

Acoustic energy can be used to cause chemical or biological agents to be transported into and/or across biological barriers, for example, in cells or tissue. Characterizing the dependence of cell membrane permeabilization on acoustic energy conditions is, however, essential to rationally designing acoustic energy protocols for pharmaceutical and other applications. Accordingly, methods are provided herein to use the quantitative dependence of cell membrane permeabilization on various acoustic parameters to enhance the transport of chemical or biological agents to be transported into and/or across biological barriers in cells or tissue, for example, to enhance delivery of drugs to cells in a specific tissue or to increase uptake of compounds which cross cell membranes poorly.

I. Acoustic Energy

As used herein, the term "acoustic energy" means any form of pressure wave, whether audible or inaudible. The frequency of the acoustic energy can be a single frequency or a combination of frequencies. The range of useful frequencies preferably is between about 1 Hz and 100 MHz, and more preferably is between about 1kHz and 10 MHz, and most preferably between 15 kHz and 2 MHz. The waveform of the acoustic energy can be of any shape, including a sinewave or a combination of sinewaves. The pressure of the acoustic energy can be up to a few hundred atmospheres, and preferably is applied at a peak positive pressure of up to 100 atmospheres. The optimal pressure is a function of acoustic frequency and other parameters described below. The acoustic energy can be applied continuously or intermittently.

Acoustic energy can be used to enhance transport by a number of different mechanisms, which broadly fall into two classes. In the first class, acoustic energy directly or indirectly (e.g., via cavitation) provides a driving force for transport. In the second class, acoustic energy increases the permeability of the biological barrier, either reversibly, partially reversibly, or irreversibly. These two mechanisms can be used independently or in combination. In the preferred embodiment, both mechanisms are used

The parameters can be selected prior to application, based on previous studies or empirical results. In a preferred method, however, feedback is obtained so that the acoustic enhancement is modified after the initial application as needed to optimize results as treatment progresses.

Acoustic Measurement Feedback

Feedback can involve measurement of one or more variables. Variables include subharmonic pressure, acoustic parameters, temperature, amount or rate of transport of molecules, extent of cavitation, and degree of permeabilization. In one embodiment, the acoustic energy or pressure is measured at one or more frequencies other than the frequency or frequencies at which the acoustic energy is applied. For example, the acoustic energy or pressure is measured at a frequency or frequencies corresponding to integer multiples of one-half or one-fourth of the frequency applied.

As described in Example 1 below, membrane permeabilization is mediated by cavitation, so that subharmonic pressure can be used as a noninvasive way to determine the degree of permeabilization resulting from exposure to acoustic energy. In addition, permeabilization caused by ultrasound should be well predicted by the acoustic parameter $\tau.P_{f/2}$, which characterizes the total cavitational exposure by accounting for both the strength of the f/2 cavitation signal and the time over which it acts.

In a preferred embodiment, the method of enhancing transport of chemical or biological agents across or into a biological barrier includes the following steps:

(a) applying acoustic energy to the biological barrier, for example, the cells or tissue, at one or more frequencies;

(b) measuring the strength of the acoustic field applied to the cells or tissue at the applied frequency or other frequencies; and

(c) using the acoustic measurement obtained in step (b) to modify continued or subsequent application of acoustic energy to the cells or tissue.

For example, in a preferred embodiment, a device applies ultrasound to a tissue and the f/2 signal is measured to assess the degree to which the tissue was permeabilized. This information can be used to estimate the

amount of drug delivered. Measurement of the f/2 signal provides a method for real-time feedback so that the ultrasound exposure based on pre-programmed or user-selected drug delivery profiles can be optimized. The technology required for both generating acoustic energy and "listening" to f/2 signals is known in the art, relatively inexpensive when mass produced, and is readily miniaturizable.

II. Chemical or Biological Agents

The methods described herein can be used to enhance transport of essentially any endogenous or exogenous chemical or biological agent for therapeutic, diagnostic, or prophylactic purposes into or across biological barriers. Useful agents include peptides, proteins, sugars, polysaccharides, nucleotides, polynucleotide molecules, and other synthetic organic or inorganic compounds. Representative proteins and peptides include hormones such as insulin, growth factors, and vaccines. Representative polynucleic acid molecules include antisense, aptamers, ribozymes, and genes, plasmids, and viral vectors. Representative synthetic organic or inorganic drugs include anti-inflammatories, antivirals, antifungals, antibiotics and local anesthetics. As used herein, the agents can be molecules or aggregates or other multi-molecular structures, including for example, virus particles or cells, liposomes or other lipid vesicles or emulsions, or particles including nano or microspheres or capsules. For direct application, the drug will typically be administered in an appropriate pharmaceutically acceptable carrier having an acoustic impedance similar to water, such as an aqueous gel, ointment, lotion, or suspension. Alternatively, a transdermal patch can be used as a carrier.

A variety of analytes are routinely measured in the body fluids such as blood, interstitial fluid, lymph, intracellular fluid or cerebral spinal fluid. Examples of typical analytes that can be measured include blood sugar (glucose), cholesterol, bilirubin, creatinine, vitamin K or other clotting factors, uric acid, carcinoembryonic antigen or other tumor antigens, and various reproductive hormones such as those associated with ovulation or pregnancy. Other analytes that can be measured include alcohol and drugs.